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Organochlorine Concentrations in Bonnethead Sharks (*Sphyrna tiburo*) from Four Florida Estuaries

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Abstract. Because of their persistence in aquatic environments and ability to impair reproduction and other critical physiological processes, organochlorine (OC) contaminants pose significant health risks to marine organisms. Despite such concerns, few studies have investigated levels of OC exposure in sharks, which are fish particularly threatened by anthropogenic pollution because of their tendency to bioaccumulate and biomagnify environmental contaminants. The present study examined concentrations of 29 OC pesticides and total polychlorinated biphenyls (PCBs) in the bonnethead shark (*Sphyrna tiburo*), an abundant species for which evidence of reproductive impairment has been observed in certain Florida populations. Quantifiable levels of PCBs and 22 OC pesticides were detected via gas chromatography and mass spectrometry in liver of 95 *S. tiburo* from four estuaries on Florida's Gulf coast: Apalachicola Bay, Tampa Bay, Florida Bay, and Charlotte Harbor. In general, OC concentrations were significantly higher in Apalachicola Bay, Tampa Bay, and Charlotte Harbor *S. tiburo* in relation to the Florida Bay population. Because the rate of infertility has been shown to be dramatically higher in Tampa Bay versus Florida Bay *S. tiburo*, the present findings allude to a possible relationship between OC exposure and reproductive health that requires further investigation. Pesticide and PCB concentrations did not appear to significantly increase with growth or age in *S. tiburo*, suggesting limited potential for OC bioaccumulation in this species compared with other sharks for which contaminant data are available. Concentrations of OCs in serum and muscle were not correlated with those in liver, indicating that these tissues are poor surrogates for measuring internal OC burden in this species via nonlethal sampling procedures.

Even at sublethal levels of exposure, organochlorine (OCs) contaminants such as pesticides and industrial chemicals pose significant health risks to aquatic organisms. Because of the ability of these compounds to interact with both invertebrate and vertebrate endocrine systems, OCs and their metabolites are capable of impairing general health and reproduction of wildlife by altering the normal function of numerous, hormone-regulated physiological processes (National Research Council 1999). Because of these hazards, there is a need to characterize levels of OCs in aquatic fauna, especially large vertebrates, because of their tendency to bioaccumulate and biomagnify environmental pollutants. In response to this need, studies have documented concentrations of OCs in a wide variety of moderate- and large-sized aquatic vertebrates including tuna (e.g., Stefanelli *et al.* 2002; Ueno *et al.* 2002), sea turtles (e.g., Alam and Brim 2000; Storelli and Marcotrigiano 2000), alligators (e.g., Guillette *et al.* 1999), crocodiles (e.g., Wu 2000a, b), sea birds (e.g., Guruge *et al.* 2001; Braune *et al.* 2002), pinnipeds (e.g., Kajiwara *et al.* 2001; Le Boeuf *et al.* 2002), walruses (e.g., Muir *et al.* 2000; Seagars and Garlich-Miller 2001), cetaceans (e.g., Kumari *et al.* 2002; Tilbury *et al.* 2002), dugongs (Vetter *et al.* 2001), and polar bears (e.g., Henriksen *et al.* 2001; Lie *et al.* 2003). As demonstrated in several of these studies, accumulation of potentially hazardous levels of OCs often occurs in large vertebrates residing in even minimally contaminated habitats. Moreover, tissue levels of these compounds have been linked with reproductive and/or health abnormalities in certain species, as well as significant declines in the size of some wildlife populations (e.g., Hutchinson and Simmonds, 1994; Crain *et al.* 1997; Tangredi and Evans 1997; Guillette *et al.* 1999; Helander *et al.* 2002; Tanabe 2002; Derocher *et al.* 2003).

Although sharks are a major component of marine megafauna, few studies have examined levels of OCs in tissues of these fish (Corsolini *et al.* 1995; Blanch *et al.* 1996; Serrano *et al.* 1997, 2000; Storelli and Marcotrigiano 2001; Storelli *et al.* 2003a, b). The relative lack of such data compared with those for other aquatic vertebrates warrants concern because many shark species often occupy terminal positions in marine food chains and, as a result, may accumulate OCs at levels similar to those in other top predators. Furthermore, the potential effects of OC exposure may have drastic implications for the health and survival of shark populations because these fish generally tend to exhibit life history characteristics consistent

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with limited reproductive potential and low rates of population growth. Given these points, it is critical to build upon previous observations on OC contamination in sharks and their relatives, especially in light of recent concerns regarding the sustainability of shark populations worldwide (Camhi *et al.* 1998).

The goal of this study was to examine OC concentrations in tissues of the bonnethead shark (*Sphyrna tiburo*), a well-characterized species for which data on reproduction (Parsons 1993a; Manire *et al.* 1995 2004; Manire and Rasmussen 1997; Manire *et al.* 1999; Gelsleichter *et al.* 2003; Nichols *et al.* 2003; Lombardi-Carlson *et al.* 2004; Gelsleichter *et al.* 2002; Chapman *et al.* 2004), feeding ecology (Cortes *et al.* 1996), growth rate (Parsons 1993b; Carlson and Parsons 1997; Lombardi-Carlson *et al.* 2004), and population growth (Cortes and Parsons 1996) are available. In addition to addressing the need for information regarding pollutant concentrations in sharks, this research was motivated by concerns that certain populations of *S. tiburo* in southwest Florida may be experiencing reproductive complications as a result of habitat degradation. In particular, high rates of infertility have been observed in *S. tiburo* residing in the Tampa Bay estuary, one of the most highly urbanized regions on Florida's Gulf coast (Parsons 1993a). In comparison, infertility rates in bonnethead shark populations inhabiting the less industrialized Florida Bay estuary were found to be significantly lower (Parsons 1993a). The present study determined concentrations of pesticides and polychlorinated biphenyls (PCBs) in liver of *S. tiburo* from these and other estuaries in southwest Florida to explore the potential relationship between OC burden and infertility rate, as well as other factors such as sex, size, and age. In addition, OC concentrations in muscle and serum of these animals were examined to determine whether they are suitable surrogates for measuring contaminant levels in sharks and their relatives via nonlethal approaches.

Materials and Methods

Sampling

Bonnethead sharks (N = 95) were collected using set gill nets between 1998 and 2001 from sites within or adjacent to four estuaries along Florida's Gulf coast (Figure 1): Apalachicola Bay (N = 9 female, 13 male), Tampa Bay (N = 17 female, 15 male), Charlotte Harbor (N = 5 female, 5 male), and Florida Bay (N = 18 female, 13 male). Studies on sediment quality in these estuaries (Seal *et al.* 1994; Cantillo *et al.* 1999; Santschi *et al.* 2001; Scott *et al.* 2002) and contaminant load in resident wildlife (Alam and Brim 2000; Brim *et al.* 2001; Oliver *et al.* 2001; Scott *et al.* 2002) indicate that they represent a wide range in the type and levels of OC exposure. Because the majority of Florida Bay lies within the boundaries of Everglades National Park, it has not been subjected to extensive urban development and remains a minimally contaminated site in comparison with other Florida estuaries. In contrast, given that it borders one of Florida's most densely populated coastal areas, Tampa Bay is generally considered to be a pollutant-impacted estuary despite recent improvements in environmental quality. Levels of OC exposure in both Apalachicola Bay and Charlotte Harbor have been reported to be intermediate between those in Florida Bay and Tampa Bay, but may have recently increased due to rapid coastal development in these areas.

Blood samples were obtained from each shark, after capture, via caudal venipuncture and were immediately placed on ice, where they were allowed to clot for 3–6 h. Blood samples were later centrifuged

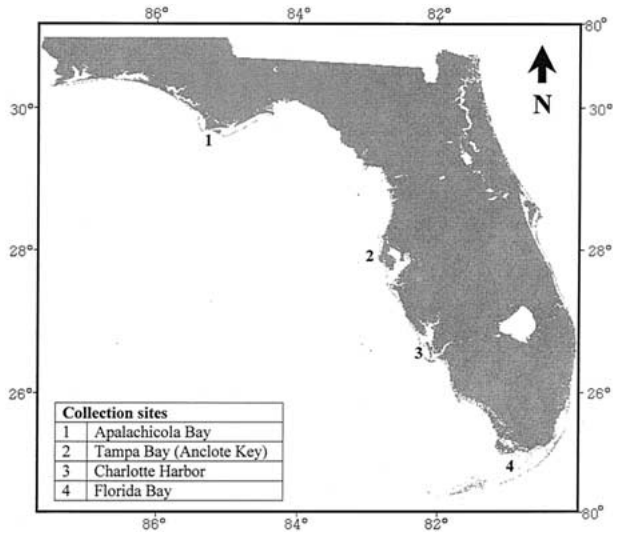

Fig. 1. Map of Florida demonstrating sampling sites used in the present study.

(1300*g*) and sera were frozen at −20°C until thawed for contaminant analysis. Sharks were sexed, measured, weighed, and transported to the laboratory on ice for sterile dissection of muscle and liver samples, which also were frozen at −20°C until processed for OC assays. Muscle samples were obtained from the left dorsolateral flank just behind the gills. Liver samples were obtained from the lower third of the right hepatic lobe. Lastly, vertebral centra from the region directly below the first dorsal fin were sampled and used to estimate age following the procedures described in Lombardi-Carlson *et al.* (2004).

Sample Extraction and Clean-up

Extraction procedures were adapted from Mill's (1959) multiresidue method for the isolation of nonpolar analytes from fatty foods. For extraction of analytes from shark muscle and liver, 10 g or the entire tissue sample was homogenized with 0.5 g of sodium sulfate and 100 mL of ethyl acetate (10 mL/g sample). The supernatant was decanted and filtered though a Buchner funnel lined with Whatman #1 filter paper and filled to a depth of 1.25 cm with sodium sulfate. The remaining homogenate was extracted and filtered a second time with filtrates combined. The pooled filtrate was concentrated until solvent-free by rotary evaporation, transferred to a 15-mL conical tube, and reconstituted in 10 mL of ethyl acetate. At this stage, the extract was either stored at −20°C or immediately subjected to clean-up procedures. A volume equivalent to 1 g of sample was removed, concentrated under dry nitrogen until solvent-free, redissolved in 2 mL of acetonitrile, vortexed for 30 s, and applied to a SPE-C_{18} cartridge (J & W Scientific, Inc., Folsom, CA) preconditioned with 3 mL of acetonitrile. The sample tube was rinsed once with 2 mL of acetonitrile, after which the rinse was applied to the SPE-C_{18} cartridge and combined with the previous eluent. The cartridge was rinsed a second time with 1 mL of acetonitrile, which also was combined with the prior eluents. The pooled extract was then applied to a 0.5-g SPE-NH_2 cartridge (Varian, Inc., Harbor City, CA) and allowed to pass under gravity with the eluent collected. The column was rinsed with 1 mL of acetonitrile, which was collected and combined with the previous eluent. Lastly, the pooled eluents were concentrated under a stream of dry nitrogen, redissolved in 300 μL of acetone, and transferred to a vial for OC analysis by gas chromatography-mass spectrometry (GC-MS).

For extraction of analytes from shark serum, 1 mL or the entire sample was mixed with 1.5 mL of acetone, vortexed for 20 s, and

Table 1. Organochlorine contaminants measured in the present study

	LOD – LOQ range (ng/g or ng/mL)		
Group/compound	Liver	Muscle	Serum
Dichlorodiphenylethanes			
p,p′-DDD	0.1 – 6	0.06 – 6	0.1 – 12
p,p′-DDE	0.1 – 3	0.6 – 3	0.1 – 6
p,p′-DDT	0.4 – 15	0.3 – 6	0.4 – 12
Methoxychlor	0.1 – 15	0.03 – 6	0.1 – 12
Chlorinated cyclodienes			
Aldrin	0.3 – 6	0.18 – 3	0.3 – 3
cis-Chlordane	0.2 – 3	0.15 – 3	0.2 – 3
trans-Chlordane	0.2 – 3	0.12 – 3	0.2 – 3
Dieldrin	0.7 – 3	0.3 – 3	0.5 – 3
Endosulfan	0.9 – 3	0.6 – 6	0.9 – 6
Endosulfan II	1.0 – 15	0.6 – 6	1.0 – 9
Endosulfan sulfate	1.2 – 15	0.6 – 15	0.9 – 12
Endrin	0.8 – 15	0.6 – 15	0.8 – 15
Endrin aldehyde	0.8 – 6	0.6 – 15	0.9 – 15
Heptachlor	0.2 – 6	0.15 – 6	0.2 – 15
Heptachlor epoxide	0.3 – 3	0.15 – 3	0.3 – 3
MC-2	0.2 – 6	0.15 – 6	0.2 – 15
MC-5	0.2 – 3	0.12 – 3	0.2 – 3
cis-Nonachlor	0.3 – 6	0.15 – 6	0.3 – 9
trans-Nonachlor	0.2 – 3	0.12 – 3	0.2 – 3
Oxychlordane	1.1 – 6	0.6 – 6	1.2 – 3
Toxaphene	200 – 7500	480 – 7500	480 – 7500
Chlorinated benzenes/cyclohexane-related compounds			
4-Bromophenylether	0.1 – 3	0.6 – 3	0.1 – 3
4-Chlorodiphenylether	0.1 – 6	0.3 – 6	0.1 – 6
α-BHC	0.4 – 15	2.1 – 6	0.3 – 15
β-BHC	0.5 – 30	0.24 – 3	0.4 – 3
δ-BHC	0.6 – 15	0.27 – 15	0.4 – 15
Hexachlorobenzene	0.1 – 30	0.06 – 15	0.1 – 15
Lindane	1.2 – 30	0.3 – 6	0.9 – 3
Other pesticides			
Mirex	0.1 – 3	0.06 – 3	0.1 – 3
Total PCBs	0.7 – 30	0.24 – 30	0.7 – 15

Note. Limits of detection (LOD) and quantitation (LOQ) for each compound are presented separately for each of the three tissue types examined.

centrifuged for 5 min at 1500*g*. The supernatant was transferred to a clean culture tube and the extraction was repeated with supernatants combined. The extract was mixed with 2 mL of methylene chloride:petroleum ether (1:1), after which the culture tube was capped, shaken for 10 s, and the contents were allowed to separate. The upper layer was transferred to a second tube and the extraction was repeated. The combined upper layers were then applied to a 5-ml SPE-Florisil cartridge (Fisher Scientific PrepSep, Fairlane, NJ), which had previously been prepared with 1.25 cm of sodium sulfate and conditioned with 10 mL of acetone:methylene chloride:petroleum ether (2:1:1). The sample was allowed to pass under gravity with the eluent collected in a conical tube. The cartridge was eluted with 4 mL of the preconditioning solvent, which was collected and combined with the first eluent. The combined extract was concentrated under a stream of nitrogen, reconstituted in 300 μL of acetone, and transferred to a vial for OC analysis by GC-MS.

Contaminant Analysis

Samples were screened for a panel of 29 pesticides and total PCBs (Table 1). Analytical grade standards for the following compounds were purchased from the sources indicated: aldrin, α-benzene hexachloride (BHC), β-BHC, Lindane, δ-BHC, *p,p′*-DDD, *p,p′*-DDE, *p,p′*-DDT, dieldrin, endosulfan, endosulfan II, endosulfan sulfate, endrin, endrin aldehyde, heptachlor, heptachlor epoxide, hexachlorobenzene, methoxychlor, mirex, *cis*-nonachlor, and *trans*-nonachlor from Ultra Scientific (Kingstown, RI); 4-bromodiphenylether and 4-chlorodiphenylether from Aldrich Chemical Co. (Milwaukee, WI); *cis*-chlordane, *trans*-chlordane, and 525, 525.1 PCB mix from Supelco (Bellefonte, PA); oxychlordane from Chem Service, Inc. (West Chester, PA); and toxaphene from Restek Corp. (Bellefonte, PA). No analytical grade standards were available for MC-2 and MC-5, isomers of heptachlor and *trans*-chlordane, respectively. Therefore, these analytes were quantified using the heptachlor and *trans*-chlordane standards.

Analysis of samples was performed using a Hewlett Packard HP-6890 gas chromatograph (Palo Alto, CA) with split/splitless inlet, operated in splitless mode. Analytes were introduced in a 1-μL injection and separated across the HP-5MS column (30 m × 0.25 mm; 0.25 μm film thickness) under a temperature program that began at 60°C, increased at 10°C/min to 270°C (at which point it was held for 5 min), then increased at 25°C/min to 300°C. Detection utilized an HP-5973 mass spectrometer in electron impact mode. Identification for all ana1ytes was conducted in full scan mode in which all ions are monitored. To improve sensitivity, selected ion monitoring was used for quantitation of all analytes, except toxaphene.

For quantitation, a five-point standard curve was prepared for each analyte ($R2 \geq 0.995$). Fresh curves were analyzed with each set of 20

Table 2. Results of multiple regression analysis of organochlorine (OC) concentrations in 95 *Sphyrna tiburo* collected from four Florida estuaries

	Factor				
Group/compound	Site	Tissue	Sex	Size	Age
Dichlorodiphenylethanes					
p,p′-DDD (*N* = 176, 159)	*P* = 0.019	*P* < 0.001	NS	NS	NS
p,p′-DDE (*N* = 176, 159)	*P* < 0.001	*P* < 0.001	NS	*P* = 0.014	NS
p,p′-DDT (*N* = 161, 144)	NS	NS	NS	NS	NS
Methoxychlor (*N* = 172, 156)	*P* < 0.001	*P* < 0.001	NS	NS	NS
Chlorinated cyclodienes					
Aldrin	NS	*P* < 0.001	NS	NS	NS
cis-Chlordane	*P* < 0.001	*P* < 0.001	NS	NS	NS
trans-Chlordane	*P* = 0.038	*P* < 0.001	NS	NS	NS
Dieldrin	*P* < 0.001	*P* < 0.001	NS	NS	NS
Endosulfan II	NS	*P* < 0.001	NS	NS	NS
Endosulfan sulfate	NS	NS	NS	NS	NS
Heptachlor epoxide	*P* < 0.001	*P* < 0.001	NS	NS	*P* = 0.010
MC-2 (*N* = 167, 150)	*P* < 0.001	*P* < 0.001	NS	NS	NS
MC-5 (*N* = 167, 150)	*P* = 0.002	*P* < 0.001	NS	NS	NS
cis-Nonachlor	*P* < 0.001	*P* < 0.001	NS	NS	NS
trans-Nonachlor	*P* < 0.001	*P* < 0.001	NS	NS	NS
Oxychlordane	*P* < 0.001	*P* < 0.001	NS	NS	NS
Other pesticides					
Mirex (*N* = 164, 148)	NS	*P* < 0.001	NS	NS	NS
Total PCBs	*P* < 0.001	*P* < 0.001	*P* = 0.047	NS	NS

Notes: Factors that significantly contributed to variations in OC concentrations are indicated by *P* value.
NS = not significant. *N* = 177 for all variables except age (*N* = 160) unless indicated otherwise in parentheses. Sample size may differ because of the inability to estimate age or measure concentrations of certain OCs because of matrix-related effects. Compounds detected in less than 5% of the total samples examined were not analyzed.

samples. Each standard and sample was fortified to contain a deuterated internal standard, 5 μL of US-108 (120 μg/mL, Ultra Scientific). All samples also contained a surrogate, 0.6 μg/g of tetrachlorometaxylene (Ultra Scientific; percentage recovery ≥66%), added at homogenization. For liver and muscle, duplicate quality control samples (nominally 0.3 or 0.75 μg/g of γ-BHC, heptachlor, aldrin, dieldrin, endrin, and *p,p′*-DDT; percentage recovery ≥60%, except aldrin, which was affected by matrix interference in some samples) were prepared and analyzed with every 20 samples. Because of limited sample, this was not possible for serum, and quality control matrices were prepared from fetal bovine serum (Hyclone, Logan, UT; percentage recovery >70%, except for dieldrin, >50%).

Data Analysis

Data for compounds detected in more than 5% of the total samples examined were analyzed using multiple regression procedures to determine whether site of capture, sex, size, age, and/or tissue type contributed to variability in OC concentrations. In individual cases when one of these compounds was not detected or was present at levels below the limit of quantitation (LOQ), numerical values of zero or the midpoint between the limit of detection and the LOQ, respectively, were assigned to permit statistical analysis. Data were subsequently grouped by factors that significantly contributed to differences in OC concentrations, and were analyzed using one-way analysis of variance (ANOVA) followed by Student–Newman–Keuls multiple comparison test. Associations between OC concentrations in liver and other tissues were examined using Pearson correlation to assess the use of muscle and/or serum as surrogates for estimating pollutant levels in *S. tiburo*. Linear regression was used to determine whether OC concentrations increased with age and growth of *S. tiburo*, an indication of contaminant bioaccumulation in this species.

Results

Quantifiable levels of 22 of the 29 OC pesticides and metabolites analyzed in the present study were detected in *S. tiburo* from at least one of the four sampling sites. Only 4-chlorodiphenylether, β-BHC, lindane, δ-BHC, heptachlor, and toxaphene were not detected in any animal, whereas hexachlorobenzene was always present in levels below the LOQ. Because 4-bromophenylether, α-BHC, endosulfan I, endrin, and endrin aldehyde were detected in less than 5% of the total samples examined, they were not subjected to further analysis. Measurable levels of PCBs were present in virtually all sharks examined, regardless of site of collection.

Multiple regression analysis of data from 177 tissue samples (*N* = 62 liver, 50 muscle, 65 serum) indicated that tissue type and site of collection were the major factors that contributed to variations in OC concentrations in *S. tiburo* from Florida's Gulf coast (Table 2). In fact, animal size and age contributed to variations in the levels of only one analyte each (*p,p′*-DDE and heptachlor epoxide, respectively). The regression model also indicated that gender was associated with variations in total PCB concentrations (male > female), but these results were marginally significant (*P* = 0.047).

Comparisons of mean pollutant concentrations by tissue type and site of capture indicated that DDT and chlordane compounds were the most abundant OC pesticides and metabolites present in *S. tiburo* from Florida estuaries (Tables 3 and 4). Other compounds that comprised a noteworthy proportion of the total OC burden included dieldrin, endosulfan II, methoxychlor, and mirex (Table 5). Although they were detected in more than 5% of the total samples examined, aldrin

Table 3. Concentrations of DDT and related compounds in liver, muscle, or serum of *Sphyrna tiburo* collected from four Florida estuaries

Site	Tissue	*N*	DDT	DDE	DDD
AB	Liver	18	19.33 ± 5.05 (13)	94.95 ± 32.97	23.80 ± 6.37
	Muscle	20	13.65 ± 3.41	4.89 ± 0.86	6.96 ± 2.25
	Serum	5	2.40 ± 2.40	BQL	9.60 ± 2.40
CH	Liver	10	23.14 ± 4.04	30.32 ± 2.82	2.48 ± 0.24
FB	Liver	16	12.30 ± 5.19 (10)	4.62 ± 1.01 (15)	2.20 ± 1.19 (15)
	Muscle	15	8.00 ± 3.28	3.73 ± 0.86	11.00 ± 3.24
	Serum	31	8.13 ± 1.02	4.75 ± 0.75	7.64 ± 1.15
TB	Liver	18	21.92 ± 2.31 (13)	26.17 ± 2.82	11.67 ± 1.75
	Muscle	15	20.11 ± 3.14	4.67 ± 0.87	5.55 ± 2.48
	Serum	29	10.34 ± 1.78	5.91 ± 0.56	9.21 ± 1.59

Notes: Values presented are means ± SE. Numbers in parentheses refer to the actual sample size used to calculate the preceding value, which occasionally differed from initial *N* because of matrix-related effects in certain samples.
AB = Apalachicola Bay, CH= Charlotte Harbor, FB = Florida Bay, TB = Tampa Bay.

Table 4. Concentrations of chlordane pesticides in liver, muscle, or serum of *Sphyrna tiburo* collected from four Florida estuaries

Site	Tissue	*N*	*trans*-Chlordane	*cis*-Chlordane	*trans*-Nonachlor	*cis*-Nonachlor	Heptachlor epoxide	MC-2	MC-5	Oxychlordane
AB	Liver	18	4.60 ± 0.96	4.35 ± 0.73	13.4 ± 2.81	13.97 ± 1.62	6.42 ± 1.19	1.84 ± 0.65	3.61 ± 0.53	11.67 ± 1.80
	Muscle	20	4.68 ± 0.99	2.66 ± 0.70	1.63 ± 0.63	0.95 ± 0.67	ND	0.93 ± 0.51	0.44 ± 0.24	ND
	Serum	5	ND	ND	0.64 ± 0.39	ND	ND but 1	ND	ND	ND
CH	Liver	10	1.58 ± 0.03	1.90 ± 0.21	11.54 ± 1.76	6.21 ± 0.92	1.46 ± 0.03	NM	NM	5.51 ± 0.80
FB	Liver	16	1.70 ± 0.59	1.65 ± 0.54	3.24 ± 0.63	8.44 ± 0.49	3.70 ± 0.95	1.32 ± 0.61	3.20 ± 0.96	ND
	Muscle	15	8.39 ± 1.22	4.76 ± 1.13	4.10 ± 1.51	3.80 ± 1.77	1.01 ± 1.02	ND	1.94 ± 0.72	ND
	Serum	31	2.62 ± 0.78	0.25 ± 0.14	1.17 ± 0.56	0.77 ± 0.54	ND	ND	ND	ND
TB	Liver	18	6.92 ± 0.82	9.17 ± 1.10	29.42 ± 2.94	22.67 ± 1.99	9.92 ± 1.09	5.36 ± 0.29	6.26 ± 0.79	16.75 ± 0.91
	Muscle	15	7.06 ± 1.11	5.03 ± 0.98	4.11 ± 1.19	2.16 ± 1.52	0.41 ± 0.41	ND	2.19 ± 0.54	ND
	Serum	29	1.72 ± 0.72	0.99 ± 0.44	9.02 ± 1.14	7.66 ± 1.20	ND but 1	ND	ND	ND

Notes: NM = not measured. Values presented are means ± SE. AB = Apalachioola Bay; CH = Charlotte Harbor; FB = Florida Bay; TB = Tampa Bay; ND = not detected.

Table 5. Concentrations of non-DDT and nonchlordane pesticides in liver, muscle, or serum of *Sphyrna tiburo* collected from four Florida estuaries

Site	Tissue	*N*	Aldrin	Endosulfan II	Endosulfan sulfate	Mirex	Dieldrin	Methoxychlor
AB	Liver	18	ND	ND	ND	9.0 ± 1.78 (16)	4.83 ± 2.10	17.28 ± 2.16
	Muscle	20	ND or BQL	14.82 ± 3.08	ND	ND	0.29 ± 0.29	2.15 ± 1.55
	Serum	5	ND or BQL	6.60 ± 4.28	ND but 1	ND	ND	9.0 ± 3.67
CH	Liver	10	ND	ND	1.00 ± 0.37	4.22 ± 0.75	4.67 ± 0.69	ND
FB	Liver	16	ND	ND	ND	6.51 ± 2.15 (9)	ND	13.61 ± 1.75 (13)
	Muscle	15	ND but 1	12.77 ± 2.64	ND	ND or BQL	0.20 ± 0.20	6.25 ± 2.92
	Serum	31	BQL	1.55 ± 0.87	1.93 ± 0.93	ND	ND	8.90 ± 1.58
TB	Liver	18	ND	ND	ND but 1	8.25 ± 1.14 (14)	3.94 ± 0.81	10.87 ± 1.79 (16)
	Muscle	15	0.91 ± 0.35	15.13 ± 3.94	ND	ND	ND	3.35 ± 1.95
	Serum	29	2.36 ± 0.66	1.86 ± 0.91	ND but 1	ND	ND	6.10 ± 1.49

Notes: NM = not measured. Values presented are means ± SE. Numbers in parentheses refer to the actual sample size used to calculate the preceding value, which occasionally differed from initial *N* because of matrix-related effects in certain samples.
AB = Apalachicola Bay; CH = Charlotte Harbor; FB = Florida Bay; TB = Tampa Bay; ND = not detected; BQL = below quantifiable levels. Compounds detected in less than 5% of the total samples examined are not presented.

and endosulfan sulfate did not contribute greatly to overall contaminant load.

Contaminants detected in more than 5% of the total samples examined were grouped by mode of action (see Table 1 for groupings) and reanalyzed to provide a condensed, but informative summary of differences in OC levels associated with site of collection (Figure 2). Significant differences in liver concentrations of dichlorodiphenylethanes (Apalachicola Bay > Tampa Bay = Charlotte Harbor > Florida Bay), chlorinated cyclodienes (Tampa Bay > Apalachicola Bay > Charlotte Harbor = Florida Bay), and total PCBs (Apalachicola Bay = Tampa Bay = Charlotte Harbor > Florida Bay) were observed among sampling areas (ANOVA and Student–Newma–Keuls, $P < 0.05$). However, these differences were not consistent for OC levels in other tissues examined, which did not vary significantly among sites with the sole exception of serum concentrations of chlorinated cyclodienes (Tampa Bay > Apalachicola Bay = Florida Bay, $P < 0.05$). Therefore, OC concentrations in muscle and serum did not appear to reflect internal contaminant load in *S. tiburo*.

The use of muscle and/or serum as surrogates for measuring levels of contamination in *S. tiburo* nonlethally was further

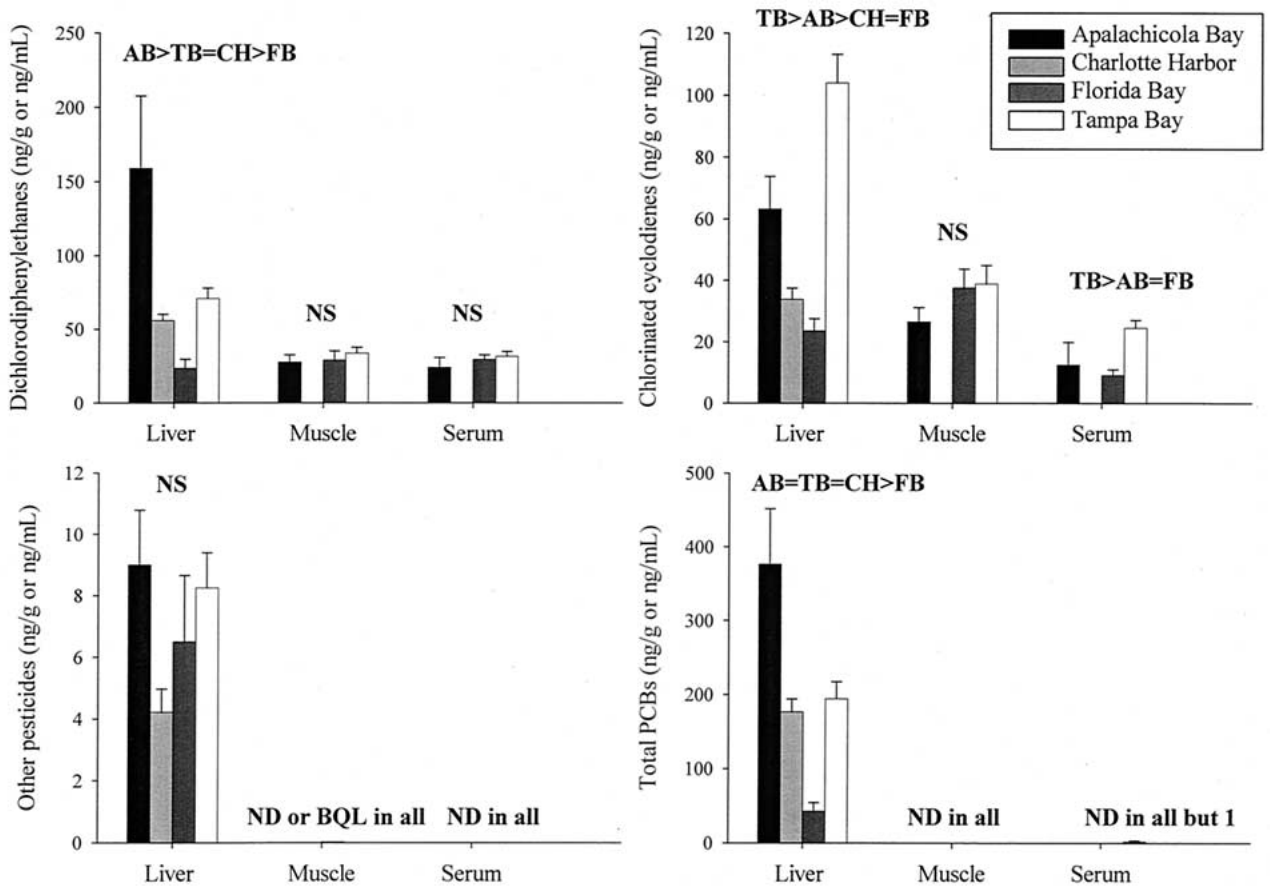

Fig. 2. Mean (±SE) concentrations of dichlorodiphenylethanes, chlorinated cyclodienes, other pesticides (mirex only, toxaphene was not detected), and total PCBs in liver, muscle, and serum of *Sphyrna tiburo* collected from four Florida estuaries. Significant differences (analysis of variance and Student–Newman–Keuls, $P < 0.05$) between sites are indicated above bars (AB = Apalachicola Bay, CH = Charlotte Harbor, FB = Florida Bay, TB = Tampa Bay). NS = not significant. Only liver was examined in Charlotte Harbor *S. tiburo*. ND = not detected: BQL = below quantifiable levels.

evaluated by performing correlation analysis on data from animals in which OC concentrations in liver and muscle ($N = 44$) or liver and serum ($N = 33$) were both tested (Table 6). Because of the lack of quantifiable levels of several OCs in muscle and serum, this was possible for only a limited number of analytes (liver–muscle: 10, liver–serum: 8). No significant correlations between OC concentrations in liver and muscle were observed. Concentrations of only two compounds in liver and serum were significantly correlated (*trans*- and *cis*-nonachlor, Pearson r, $P < 0.05$), but the proportion of explained variance for these associations was low ($R^2 = 0.16$ and 0.36, respectively).

Because of significant differences in contaminant levels among sites, relationships between liver OC concentrations and age or size were examined separately for each sampling area (Table 7). Of the 101 regression analyses performed, only 19 data sets exhibited a significant linear relationship between OC concentrations and age or growth of *S. tiburo* ($P < 0.05$). Only 10 of these data sets reflected an increase in OC levels over time, and all but 1 were associated with rather poor fit around the regression line ($R^2 < 0.75$). Taken together, these results indicated limited potential for bioaccumulation of OCs in *S. tiburo*.

Table 6. Correlations between organochlorine concentrations in liver and muscle ($N = 44$) or liver and serum ($N = 33$) of individual *Sphyrna tiburo*

Compound	Liver – Muscle ($N = 44$)	Liver – serum ($N = 33$)
p,p′-DDD	NS (43)	NS
p,p′-DDE	NS (43)	NS
p,p′-DDT	NS (32)	NS (23)
Methoxychlor	NS (39)	NS (29)
cis-Chlordane	NS	NS
trans-Chlordane	NS	NS
MC-5	NS	—
cis-Nonachlor	NS	$P = 0.002$, $R^2 = 0.36$
trans-Nonachlor	NS	$P = 0.019$, $R^2 = 0.16$
Mirex	NS (31)	—

Notes. Values that are significantly correlated are indicated by P value and the coefficient of determination (Pearson r).
NS = not significant. Numbers in parentheses refer to the actual sample size for analysis, which occasionally differed from initial N because of matrix-related effects in certain samples.

Table 7. Results of linear regression analyses used to characterize relationships between organochlorine concentrations and size or age of *Sphyrna tiburo*

	Total length				Age		
Compound	AB (N =18)	CH (N = 10)	FB (N = 16)	TB (N = 18)	AB (N = 17)	FB (N = 15)	TB (N = 18)
p,p′-DDD	NS	NS	NS (15)	NS	NS	NS (14)	NS
p,p′-DDE	*P = 0.003 (13)*[a]	NS	NS (15)	NS	*P < 0.001*[b]	NS (14)	NS
p,p′-DDT	NS	NS	NS (10)	NS (13)	NS (12)	NS (9)	NS (13)
Methoxychlor	NS	—	NS (13)	NS (16)	NS	NS (13)	NS (16)
cis-Chlordane	NS	NS	NS	NS	NS	NS	NS
trans-Chlordane	*P* = 0.009	NS	NS	NS	*P* = 0.027	NS	NS
Dieldrin	NS	NS	—	NS	NS	—	NS
Endosulfan sulfate	—	NS	—	—	—	—	—
Endrin aldehyde	—	—	NS	—	—	NS	—
Heptachlor epoxide	*P* = 0.010	NS	NS	NS	*P* = 0.012	NS	NS
MC-2	NS	—	NS	NS	NS	NS	NS
MC-5	NS	—	NS	NS	NS	NS	NS
cis-Nonachlor	NS	*P = 0.038*[a]	NS	NS	NS	*P* = 0.014	NS
trans-Nonachlor	*P = 0.043*[a]	*P = 0.020*[b]	*P* = 0.035	NS	NS	*P* = 0.018	NS
Oxychlordane	NS	NS	—	NS	NS	NS	NS
Mirex	*P = 0.008 (16)*[a]	NS	NS	NS (14)	*P < 0.001 (15)*[c]	NS (9)	NS (14)
Total PCBs	*P < 0.001*[b]	*P* = 0.013[b]	NS	*P* = 0.047	*P < 0.001*[b]	NS	*P* = 0.026

Notes: Pesticides that significantly increase in concentration with size or age are indicated by italicized *P* values, whereas nonitalicized values represent significant negative slopes (analysis of variance).

[a] $R^2 < 0.50$.

[b] $R^2 = 0.50$–0.75.

[c] $R^2 = 0.75$.

NS = not significant. Numbers in parentheses refer to the actual sample size used for analysis, which occasionally differed from initial *N* because of matrix-related effects in certain samples.

Discussion

The present study represents one of the largest efforts to characterize levels of OC contamination in an elasmobranch species to date. It also is one of few studies that have investigated OC exposure in coastal sharks, many of which face unique risks from marine pollution because of their dependence on increasingly degraded nearshore and estuarine areas as sites for breeding and juvenile refuge (Castro 1993). In fact, all previously published studies on OC contamination in elasmobranchs have focused on deep-water benthic or pelagic species (Corsolini *et al.* 1995; Blanch *et al.* 1996; Serrano *et al.* 1997, 2000; Storelli and Marcotrigiano 2001; Fisk *et al.* 2002; Storelli *et al.* 2003a, b) with the exception of recent observations on leopard sharks (*Triakis semifasciata*) from San Francisco Bay, CA (Davis *et al.* 2002). Because most of these studies have documented the ability of sharks to accumulate OCs at potentially hazardous levels, it was important to determine whether similar or greater risks are experienced by coastal species like *S. tiburo*, which may inhabit pollutant-impacted regions on a more regular basis.

Despite prolonged residence in historically contaminated regions, *S. tiburo* does not appear to accumulate OCs at levels that have been reported in deep-water elasmobranchs. For example, liver concentrations of total DDTs in *S. tiburo* rarely exceeded 100 ng/g, a quantity far below those observed in Arctic Sea Greenland sharks, *Somniosus microcephalus* (mean ± SE = 7159 ± 1271 ng/g [lipid basis], Fisk *et al.* 2002), and Mediterranean Sea gulper sharks, *Centrophorus granulosus*, and longnose spurdogs, *Squalus blainvillei* (mean ± SE = 4481 ± 961 and 1625 ± 439, respectively: Storelli and Marcotrigiano 2001). Total chlordane concentrations in the liver of *S. microcephalus* (mean ± SE = 1815 ± 473 ng/g [lipid basis], Fisk *et al.* 2002) also surpassed those detected in *S. tiburo* by a considerable margin. In addition, total PCB concentrations in *S. tiburo* liver were far less than those observed in a number of species including *S. microcephalus* (mean ± SE = 3442 ± 650 ng/g [lipid basis], Fisk *et al.* 2002), *C. granulosus* (mean ± SE = 1741 ± 531, Storelli and Marcotrigiano 2001), *S. blainvillei* (mean ± SE = 958 ± 658, Storelli and Marcotrigiano 2001), blackmouthed dogfish, *Galeus melastomus* (mean ± SE ranging from 853 ± 471 to 1072 ± 387 ng/g, Storelli *et al.* 2003b), and 7 of 8 squaloid dogfish species from the northwest coast of Africa (mean ± SE ranging from 387 ± 127 to 4723 ± 3,670 ng/g; Serrano *et al.* 2000). Although total DDT and PCB concentrations in adipose fat of blue (*Prionace glauca*) and thresher (*Alopias vulpinus*) sharks from Italy's Mediterranean coast were generally comparable with those observed in the present study, these species also appear to be capable of accumulating much greater levels of OCs than, *S. tiburo* (values ranged from 14 to 300 ng/g and 70 to 4400 ng/g for total DDTs and PCBs, respectively; Corsolini *et al.* 1995).

In contrast with that in deep-water elasmobranchs, OC concentrations in leopard sharks from San Francisco Bay were generally lower than those observed in *S. tiburo*. In fact, liver concentrations of total DDTs, chlordanes, and PCBs in *S. tiburo* from even the least contaminated sites on Florida's Gulf coast were greater than those observed in *T. semifasciata* (median = 5.3 ng/g, 1.1 ng/g, and 11 ng/g for total DDTs, chlordanes, and PCBs, respectively; Davis *et al.* 2002). Liver concentrations of dieldrin in *T. semifasciata* (median = 0.2 ng/g; Davis *et al.*

2002) also were lower than those detected in *S. tiburo* with the exception of animals residing in the Florida Bay estuary. Nonetheless, differences in OC concentrations in these two species were relatively minor in comparison with those between *S. tiburo* and the deep-sea sharks previously discussed.

The large degree of disparity between OC concentrations in deep-water sharks and coastal species such as *S. tiburo* and *T. semifasciata* is likely a consequence of differences in the feeding behavior, longevity, and/or lipid content of these animals. Unlike coastal sharks, which generally feed on benthic invertebrates and small fish (Cortés *et al.* 1997; Vebber and Cech 1998), some deep-water species such as *S. microcephalus* consume prey as large as marine mammals (Fisk *et al.* 2002), making them more likely to biomagnify OCs and other environmental contaminants. Furthermore, although they are difficult to age (MacFarlane *et al.* 2002), deep-sea sharks are generally considered to be more long-lived than coastal species. Therefore, even moderately sized deep-water sharks have the potential to bioaccumulate significant concentrations of OCs over time. Following this premise, the relatively short lifespan of *S. tiburo* (Lombardi *et al.* 2004) may explain the comparatively limited ability of bonnethead sharks to accumulate greater concentrations of OCs as they age. Surprisingly, Serrano *et al.* (2000) and Fisk *et al.* (2002) also found no relationship between OC concentrations and growth in Portuguese dogfish (*Centroscymnus coelolepis*) and *S. microcephalus*, respectively, but these findings were likely due to limited range in the size of specimens.

Variations in pesticide concentrations of *S. tiburo* from different Florida estuaries appeared to reflect what is known regarding levels of OC contamination in these regions. With few exceptions, the concentrations of individual pesticides were highest in bonnethead sharks from Tampa Bay, a site generally considered to be the most pollutant-impacted estuary on Florida's Gulf coast. Previous studies have reported concentrations of OC pesticides in Tampa Bay sediments that exceed guidance values for adverse biological effects (Seal *et al.* 1994; Carr *et al.* 1996; Santschi *et al.* 2001; Macauley *et al.* 2002), as well as substantial accumulation of these same compounds in resident wildlife (i.e., oysters; Fisher *et al.* 2000; Oliver *et al.* 2001). Concentrations of dichlorodiphenylethanes in particular also were high in Apalachicola Bay sharks, a finding seemingly at odds with reports of only moderate levels of OC contamination in this region (Seal *et al.* 1994). However, more recent studies have demonstrated that levels of certain pesticides (e.g., DDD, DDE) in wildlife (i.e, oysters, striped bass, sea turtle eggs) from Apalachicola Bay may rival or even exceed those from Tampa Bay (Alam and Brim 2000; Brim *et al.* 2001; Oliver *et al.* 2001). Although pesticide concentrations were generally low in Charlotte Harbor *S. tiburo*, the levels of total DDTs in these animals also were similar to those observed in Tampa Bay sharks and justify additional investigations on the poorly studied contaminant levels in this estuary.

Overall pesticide concentrations were lowest in Florida Bay *S. tiburo*, a finding that was expected based on the low degree of OC contamination in this region (Seal *et al.* 1994). However, it was surprising that sharks from Florida Bay did not contain detectable tissue concentrations of endosulfans, the OC pesticides of greatest concern in this estuary because of their prevalent utilization on south Florida vegetable crops (Scott *et al.* 2002). Low accumulation of these compounds in *S. tiburo* may be associated with diet, given that earlier studies have detected considerably higher concentrations of endosulfans in herbivorous and omnivorous teleosts when compared with carnivorous species (Scott *et al.* 2002).

Although their use for industrial applications was banned in the United States in the late 1970s, PCBs remain abundant in the aquatic environment because of their persistence in marine sediments and continued use in other countries (National Research Council 1999). Because of this, the presence of detectable concentrations of PCBs in virtually all *S. tiburo* that were examined in this study was not unexpected. As for most pesticides, concentrations of PCBs were low in Florida Bay *S. tiburo*, presumably because of limited historical use in this region. Tissue concentrations of these compounds were significantly greater in sharks from all other sampling areas, but did not differ among these sites. These results were generally consistent with recent studies on OC concentrations in oysters from Apalachicola Bay and Tampa Bay, which suggested comparable levels of PCB contamination in these estuaries (Oliver *et al.* 2001). Although moderately elevated concentrations of PCBs in Apalachicola Bay, Tampa Bay, and Charlotte Harbor sharks draw attention to the possible health risks associated with these compounds, it is difficult to address such concerns because of the lack of specific data regarding tissue levels of the highly toxic, "dioxin-like" coplanar PCBs in the present study. However, because potentially hazardous concentrations of some of the most toxic coplanar PCBs (e.g., PCBs 77, 118, 126) have been detected in Apalachicola Bay and Tampa Bay wildlife (e.g., oysters, sea turtles; Alam and Brim 2000; Oliver *et al.* 2001), it is reasonable to consider that these compounds constitute a nontrivial proportion of the total PCB burden in sharks residing in these same areas. Therefore, more detailed investigations on PCB contamination in Florida shark populations are needed.

In a study on geographic variation in reproduction of Florida *S. tiburo*, Parsons (1993a) reported that Tampa Bay populations of this species experience markedly higher rates of infertility in comparison with those residing in the Florida Bay estuary. Because infertility is generally rare in elasmobranchs (Parsons 1983; Hanchet 1988), Parsons (1993a) hypothesized that differences in the reproductive success of these populations may be associated with environmental factors such as anthropogenic pollution. The results of the present study add some weight to this notion by demonstrating that Tampa Bay sharks are exposed to and accumulate greater concentrations of OC contaminants than more reproductively fit Florida Bay *S. tiburo*. Nonetheless, evidence for a relationship between OC exposure, and reproductive impairment in this species is circumstantial at best. Comparisons of reproductive fitness in populations of *S. tiburo* from the four study sites are being conducted to address these concerns, and published accounts of this research are forthcoming.

Although modest amounts of blood can be obtained from elasmobranchs using nonlethal procedures, the results of the present study do not support the use of serum for assessing internal OC concentrations in these fishes. Serum pesticide concentrations in *S. tiburo* generally were not correlated with those in liver, the tissue most likely to accumulate OCs in elasmobranchs because of its high lipid content. In fact, many OC contaminants present in liver of *S. tiburo* were not detected in serum from the same individuals. Concentrations of OCs in muscle of *S. tiburo* also were not correlated with those in liver,

and similarly are not recommended as indicators of internal contaminant load. However, assessments of muscle OC concentrations in sharks have merit for characterizing the risks that aquatic contaminants pose to human populations that consume meat from these fish.

In summary, the present study has provided a wealth of novel data regarding levels of OC contamination in coastal shark populations residing in Florida estuaries. Although *S. tiburo* does not appear to accumulate OCs at concentrations observed in certain deep-water sharks, the potential relationship between contaminant exposure and reproductive failure in this species warrants further investigations on the effects of OCs on sharks and their relatives.

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